

## Advanced Specimen Collection and Culture Workup

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## Objectives

At the completion of this program the participants will be able to:

- Review collection and transport procedures for blood, urine, CSF, and other sterile fluid specimens submitted for microbiological culture.
- Summarize appropriate algorithms for culture workup of blood, urine, CSF, and other sterile fluid specimens.
- Correlate culture types with clinical relevance

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## Role of the Microbiology Laboratory in Patient Care

- To analyze and manage specimens
- To communicate effectively with healthcare professionals involved, both before and after specimen analysis

J. M. Miller, 1998 MLO 6: 28-34

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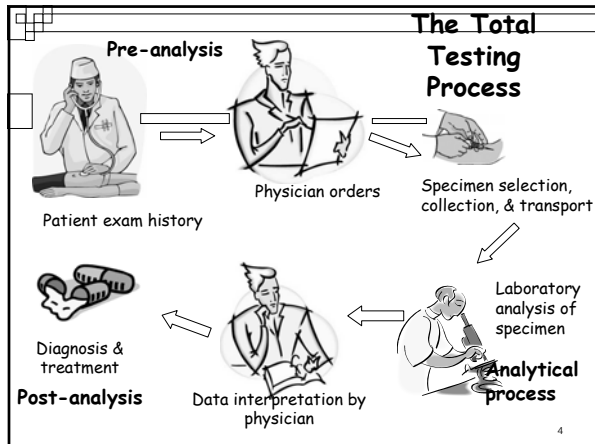
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## Specimen Selection

- The specimen must be material from actual infection site and must be collected with a minimum of contamination from adjacent tissues, organs, or secretions
- Optimal times for specimen collection must be established for the best chance of recovery of causative microorganisms.

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## Specimen Collection

- A sufficient quantity of specimen must be obtained to perform the culture techniques requested.
- Appropriate collection devices, specimen containers, and culture media must be used to ensure optimal recovery of microorganisms
- Cultures must be obtained prior to the administration of antibiotics
- The culture container must be properly labeled
- Provide complete information on specimen requisition forms

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# Specimen Transport Systems

- Sterile screw-cap cups, petri dishes, tubes
- Swabs
  - Swab Transport system
  - Calcium Alginate Swabs
  - Cotton Swabs
  - Dacron Swabs
  - Nasopharyngeal-urethrogenital swabs
- *N. gonorrhoeae* transport systems
- Proprietary swab systems for molecular testing for GC/CT
- Anaerobic Transport Systems
- Viral Transport Systems

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Organism	% Survival from 0 h count (100%)					
	PAC		EZ		CAG	
	24 h	48 h	24 h	48 h	24 h	48 h
<i>Neisseria gonorrhoeae</i>	<1	0	0	0	23	6
<i>Haemophilus influenzae</i>	<1	0	<1	0	84	24
<i>Streptococcus pneumoniae</i>	18	4	<1	0	13	<1
<i>Streptococcus pyogenes</i>	113	129	2	<1	76	53
% Avg recovery	33	33	1	0	49	22

PAC - Port-A-Cul (Becton Dickinson, Cockeysville, Md.)  
EZ - Culturette EZ (Becton Dickinson, Cockeysville, Md.)  
CAG - Copan Venturi Transystem Amies gel without charcoal (Copan Diagnostics, Caron, CA)

Perry, J.L. 1997. Assessment of swab transport systems for aerobic and anaerobic organism recovery. J. Clin. Microbiol 35:1269-1271

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Perry, J.L. 1997. Assessment of swab transport systems for aerobic and anaerobic organism recovery. J. Clin. Microbiol 35:1269-1271

# Quality Control Of Microbiological Transport Systems

- NCCLS document – M40 A
- Describes criteria to consider when choosing a microbiological transport device
- Presents quality control guidelines for both the manufacturer and the testing laboratory
- Provides a method by which laboratories can validate the manufacturer's claims and compare devices

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## CAP CHECKLIST

### ■ Question MIC.11030 PHASE: II

- Is there a documented procedure describing methods for patient identification, patient preparation, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing, consistent with good laboratory practice?

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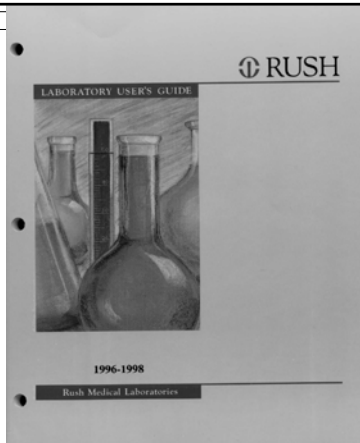
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### ■ User guides

### ■ Virtual user guides or procedure manuals



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## Web-based User's Guide

### ■ Benefits

- Users can access information with less effort.
- More accessible information venues (internet and intranet)
- Providing accurate and up to date information saves time, effort and money in communicating changes and information to the staff

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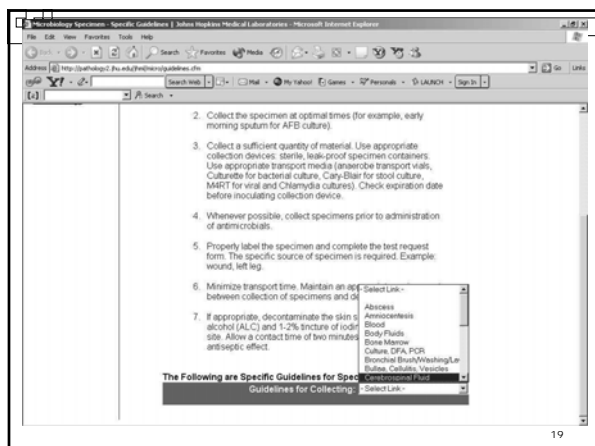
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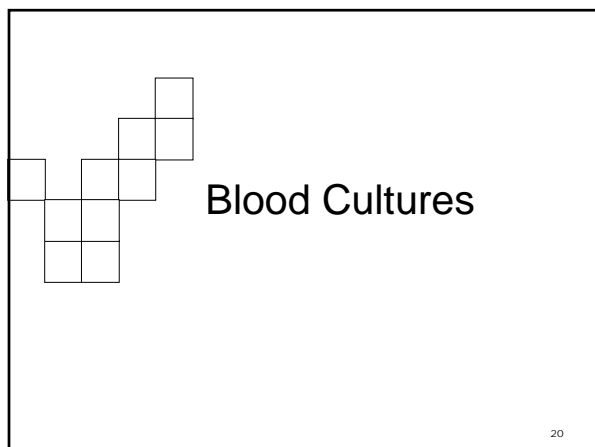
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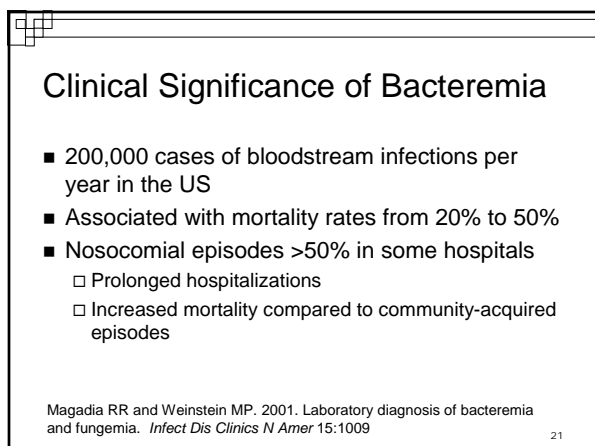
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# Bacteremias

- Blood cultures are the gold standard for diagnosing a bacteremia
- Detecting the presence of bacteria in the blood is one of the most important functions in the clinical microbiology laboratory

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- [illegible]

# Diagnostic and Prognostic Importance of Positive Blood Cultures

- **Diagnostic**
  - Establishes infectious etiology for patient's illness
  - Provides organism for susceptibility testing and optimization of antimicrobial therapy
- **Prognostic**
  - Provides evidence of failure of host defenses to contain infection at primary site
  - Provides evidence of failure of physician to remove, drain, or otherwise adequately treat primary infection

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- [illegible]

# Blood Cultures: Key Elements

- Timing
- Skin Antisepsis
- Collection
- Number
- Volume
- Choice of blood culture media
- Duration of Incubation
- Special pathogens
- Quality assurance

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## Timing of Blood Cultures

- Optimal time
  - Just before the onset of a shaking chill
- Fever detected
- General rule
  - Collect 2 BC simultaneously
- Li et. al. (JCM 1994; 32:2829-2831) found that the interval between blood cultures was not clinically important

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## Common Antiseptic Agents

	Mechanism of Action	Rapidity of Action	Residual Effect	Affected by Organic Matter	Primary Use
2% chlorhexidine gluconate/70% isopropyl alcohol	Denature protein & disrupt cell membrane	Rapid	Excellent	Efficacy not affected by organic matter	Skin prep
Iodophors	Substitution by free iodine	Intermediate	Minimal	Diminished efficacy by organic matter	Surgical hand scrub, handwash & skin prep
Alcohol	Denature proteins	Rapid	None	No data	Surgical hand scrub, handwash & skin prep
Tincture of Iodine (2%)	Denature proteins & substitution by free iodine	Rapid	Minimal	No data	Skin prep

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## Comparison of Antiseptic Agents

Table 2. Microorganisms That Were Recovered and Classified as Contaminants or as True Pathogens

Microorganism	Iodine-iodine Group		Chlorhexidine Group	
	Contaminants (Patients)	True Pathogens (Patients)	Contaminants (Patients)	True Pathogens (Patients)
---(7)---				
Coxsack-negative staphylococci	36 (33)	10 (8)	16 (14)	6 (4)
Staphylococcus aureus	0	7 (4)	0	9 (5)
Streptococcus species	0	7 (4)	1 (1)	6 (3)
Enterococcus faecalis	0	1 (1)	0	1 (1)
Escherichia coli	0	3 (2)	0	6 (4)
Klebsiella pneumoniae	0	1 (1)	0	4 (2)
Pseudomonas aeruginosa	0	3 (2)	0	2 (2)
Acinetobacter baumannii	0	1 (1)	0	1 (1)
Anaerobic organisms	0	0	0	1 (1)
Acromobolus species	0	2 (1)	0	4 (1)
Candida species	0	2 (2)	0	2 (1)

Mimoz, O et. al. 1999. Chlorhexidine compared with povidone-iodine as skin preparation before blood culture. A randomized, controlled trial. *Ann Intern Med.* Dec 7;131(11):834-7

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## Skin Antisepsis

- After palpitation, scrub the venipuncture site with 70% alcohol for a minimum of 30 s.
- Apply antiseptic agent in concentric circles away from the puncture site covering a circular area 1.5 to 2 in. in diameter



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## Blood Collection

- Methods
  - Needle and syringe
  - Butterfly draw
  - Direct draw
    - Vacutainer-type
    - Needle transfer devices
  - Aspiration from IV catheters
    - Increasing use
    - Increased contamination rates
      - If done, also obtain peripheral BC to validate

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## BLOOD CULTURES

- Definition
  - Blood obtained from one venipuncture site defines one blood culture, regardless of the number of bottles filled
  - Highly dependent on the collection technique for its sensitivity and specificity

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## Optimal Blood Volume

- Most important variable for improving detection of bacteremia and fungemia
- Number of microorganisms present in blood
  - Adults- <1 to 10 CFU/ml
  - Pediatric – 100 to 1000 CFU/ml
- Recommended volume for adults
  - 20 to 30 ml per venipuncture

Magadia RR and Weinstein MP. 2001. Laboratory diagnosis of bacteremia and fungemia. *Infect Dis Clinics N Amer* 15:1009

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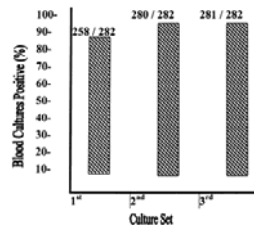
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## Number of Blood Cultures

- Rate of positivity increases (up to a point) as more cultures are obtained
- Detection of etiologic agent
  - 1<sup>st</sup> BC – 91.5%
  - 2<sup>nd</sup> BC – 99.3%
  - 3<sup>rd</sup> BC – 99.6%



Weinstein, M.P., et. al. 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. *Rev. Infect. Dis.* 5:35-53

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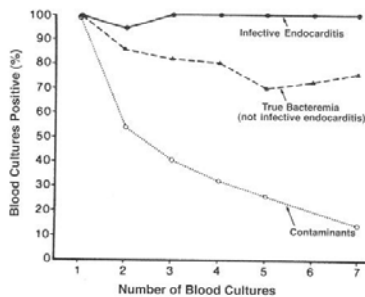
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## Diagnostic Importance of Separate Blood Cultures



Weinstein, M.P., et. al. 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. *Rev. Infect. Dis.* 5:35-53

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## True Bacteremia vs. Contamination

Clinical significance of <i>Staphylococcus epidermidis</i> isolates from blood cultures				
# of sets positive	# of sets obtained	% Significant	% Contaminate	% Indeterminate
1	1	0	97	3
1	2	2	95	3
2	2	60	3	37
1	3	0	100	0
2	3	75	0	25
3	3	100	0	0

Weinstein, M.P., et. al. 1997. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin. Infect. Dis.* 24:584-602

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## Volume of Blood Sampled

Patient wt (lb)	Recommended blood vol/culture (ml)	Total blood vol for 2 cultures (ml)	Vol of blood equal to 1% of patient's total blood vol (ml)
<19	1	2	2
18-30	3	6	6-10
30-60	5	10	10-20
60-90	10	20	20-30
90-120	15	30	30-40
>120	20	40	>40

Kaditis, A.G., et. al. 1996. Yield of positive blood cultures in pediatric oncology patients by a new method of blood culture collection. *Pediatr. Infect. Dis. J.* 15:615-620

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## CAP CHECKLIST

- Question MIC.22630 PHASE: II
  - ☐ Are sterile techniques for drawing and handling of blood cultures defined, made available to individuals responsible for specimen collection, and practiced?
- Question MIC.22640 PHASE: I
  - ☐ Are adequate volumes of blood collected for detection of sepsis?

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## Choice of Blood Culture Media

- Several commercially available blood culture systems
  - Two bottle blood culture sets
    - Aerobic and anaerobic
  - Blood both ratio (1:5)
  - Inactivation or binding of antimicrobials
    - Resin, activated charcoal
      - Detects more contaminants, more expensive

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## Duration of Incubation

### Continuous-monitoring blood culture systems

- Five-6 day protocols are acceptable for majority of pathogens (BacTAlert and BACTEC series)<sup>1</sup>
- Four day protocols suggested for ESP system, BACTEC 9240<sup>2-4</sup>
- Terminal subcultures are not required for any system
- Prolonged incubation periods (or alternative systems) may be required for fastidious pathogens

1 Hardy DJ, et. al. 1992. Time to detection of positive BacTAlert blood cultures and lack of need for routine subculture of 5-to-7-day negative cultures. *J Clin Microbiol* 30:2743.

2 Doern GV, et. al. 1997. Four-day incubation period for blood culture bottles processed with the Difco ESP blood culture system. *J Clin Microbiol* 35:1290.

3 Han XY and Truant AL. 1999. The detection of positive blood cultures by the AccuMed ESP-384 System: The clinical significance of three-day testing. *Diagn Micro Infect Dis* 33:1.

4 Johnson AS, et. al. 2000. Four day incubation for detection of bacteremia using the BACTEC 9240. *Diagn Microbiol Infect Dis* 38: 195-9

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## Extended Incubation for Presumptive Endocarditis

- Many labs are doing this
- Limited data on utility
- Rush unpublished data---no meaningful information
- Not needed for HACEK group detection

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## Multicenter Study of Extended Incubation for HACEK Organisms

Study site	Total positive blood cultures	Number of cultures positive for HACEK	HACEK Species Isolated	Time to detection (days)
JHH	6519	7	<i>A. actinomycetemcomitans</i> (1) <i>E. corrodens</i> (1) <i>C. hominis</i> (2)* <i>H. parainfluenzae</i> (3)	5 4 3 3-5
ARUP	2301	1	<i>H. parainfluenzae</i> (1)	4
RWJUH	1462	1	<i>H. parainfluenzae</i> (1)	4
TOTAL	10,282	9 (0.08%)		4 days (mean duration)

Bhally HS, et. al. 2004. Abstracts of the 42<sup>nd</sup> Annual Meeting of IDSA, Boston, MA, #453, p.120

## Quality Assurance

- Monitor contamination rates
  - Workup and reporting leads to over-utilization of antibiotics and other associated costs
- Monitor volume
  - Low volumes may decrease sensitivity
  - When < 10 ml available fill aerobic bottle only
- Monitor positivity rates
  - Infection control issues
  - Contamination issues
- Monitor solitary blood cultures
  - May compromise care due to poor sensitivity

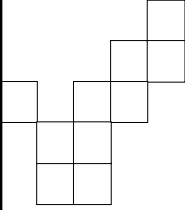
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## Blood Culture Contamination

- Definition
  - Typical skin pathogens
    - *viridans* strep, *Corynebacterium* sp., *Bacillus* sp., coagulase neg staph, *P. acnes*
  - Number of positive and negative cultures in an episode
  - Results of concurrent microbiology tests
  - Compatibility of clinical features with typical features of infection
- Rates should be  $\leq 3.0\%$

Weinstein MP. 1997. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin Infect Dis* 24:585-602

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## Cerebrospinal Fluid Specimens

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
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## Acute Meningitis: Etiologic Agents

- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Listeria monocytogenes*
- *Streptococcus agalactiae*
- *Haemophilus influenzae*
- *Staphylococcus aureus*
- Gram negative bacilli
- Anaerobes
- Amoeba

Thomson RB, Bertram H. 2001. Laboratory diagnosis of central nervous system infections. *Infect Dis Clinics N America* 15:1047.

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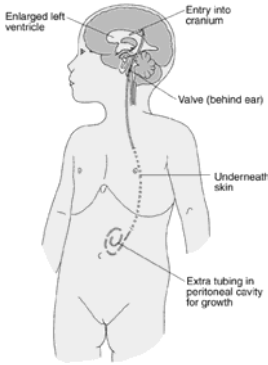
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## Ventriculoperitoneal Shunt Placement



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## Acute Meningitis: Shunt Related

- Coagulase-negative staphylococci
- *Staphylococcus aureus*
- *Propionibacterium acnes*
- Gram negative enteric bacilli
- Non-fermenting gram negative bacilli

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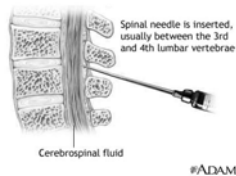
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## CSF Collection



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## CSF Collection and Processing

### Major Pitfalls and Controversies

- ☐ Failure to properly decontaminate
- ☐ VOLUME
- ☐ Timely transport
- ☐ Pre treatment with antibiotics
- ☐ Bacterial antigen detection tests
- ☐ Media
- ☐ Duration of incubation

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## CSF Antigen Detection Revisited

- First and second generation “BAD” tests
  - Poor sensitivity and specificity
  - Results do not alter therapy
  - Use selectively based upon lab practice
- New assay for *S. pneumoniae*
  - NOW *S. pneumoniae* Urinary Antigen Test (Binax, Inc. Portland, ME)
  - Immunochromatographic membrane assay—detects C polysaccharide cell wall antigen

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## NOW® *Streptococcus pneumoniae* Assay for CSF

Samra Z, et. al. 2003. *Diagn Microbiol Infect Dis* 45:237.

- Study performed in 900 bed children's hospital in Israel
  - 519 pts. with suspected meningitis were enrolled
  - CSF and blood obtained concomitantly
  - CSF and urine samples obtained for NOW testing
- Results
  - Pos CSF antigen from 21/22 pts. with pneumococcal meningitis (sens 95.4%); pos urine antigen 12/22 pts.; Gram stain 68.2% sensitive
  - Neg CSF antigen from all 27 pts with other pathogens; 5 false pos urine antigens
  - All 470 pts with no pathogens recovered were CSF antigen neg; 63 were urine antigen pos

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## CSF Specimens: Processing

- Process within 1-2 hr of collection
- Centrifuge volumes > 0.5 ml to concentrate pathogens
- Treat as “stat” for Gram stain performance and interpretation
  - Cytocentrifugation
  - Pos stain: microbiology “critical value”
- Inoculate sediment to:
  - 5% sheep blood agar, chocolate agar incubate in 5-10% CO<sub>2</sub> x 72 h
  - Include broth for patients with shunts and infection adjacent to subarachnoid space; incubate x 5-7 days

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
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## Acute Bacterial Meningitis

### Use of Gram Stain

- Dunbar SA, et. al. 1998. *J. Clin. Microbiol* 36: 1617
  - 2653 adult CSF specimens
    - 56 positive for *C. neoformans*, *S. pneumoniae*, or *N. meningitidis*
    - 88% had a positive stain result
      - If patients with prior antibiotic therapy excluded
        - CSF Gram stain - 92% sensitive

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
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## CSF Broth Cultures:

### Evidence Against Routine Use

- Lessing MP, et.al. 1996. *Eur J Clin Microbiol Infect Dis* 15:79.
- Morris AJ, et. al. 1995. *J Clin Microbiol* 33:161.
  - Only 2/88 broth only isolates were clinically significant
  - Continued use of broth recommended only for shunt infections
- Sturgis CD , et. al. 1997. *Am J Clin Pathol* 108:217.
- Dunbar SA, et. al. 1998. *J Clin Microbiol* 36: 1617
  - 82% of pathogens recovered on both solid media and in broth
  - 220 contaminants; 55% broth only
  - Exclusion of broth would have missed no acute bacterial meningitis cases, but 25% of shunt assoc cases

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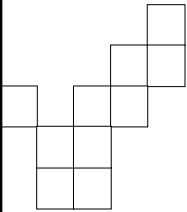
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## Sterile Body Fluids

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## Inoculation of Sterile Body Fluids to Blood Culture Bottles

- Inoculate blood culture bottles at bedside when volume is sufficient
  - Minimum of 1 mL of specimen/bottle
  - Collect aerobic and anaerobic bottle set
  - BacT/Alert FAN bottles outperformed conventional media and standard bottles in several studies
- Send additional fluid in sterile container for immediate Gram's stain and culture for Mycobacteria, fungus or "special" pathogens
- Most useful for synovial fluids and peritoneal fluid/CAPD; literature mixed for pleural fluid

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## Inoculation of Sterile Body Fluids to Blood Culture Bottles

- Advantages
  - Simplifies specimen processing
  - Shortens time to detection
  - Enhances recovery of fastidious pathogens
- Disadvantages
  - Contamination rates increase

Simor AE, et. Al. 2000. Evaluation of the BacT/Alert microbial detection system with FAN aerobic and FAN anaerobic bottles for culturing normally sterile body fluids other than blood. *Diagn Microbiol Infect Dis* 37:5.

Bourbeau P, et. Al. 1998. Use of the BacT/Alert blood culture system for culture of sterile body fluids other than blood. *J Clin Microbiol* 36: 3273.

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## Inoculation of Synovial Fluid to Blood Culture Bottles

- Enhances recovery of fastidious and slow growing pathogens eg. *Kingella kingae*
  - Yagupsky P. 1992. *J Clin Microbiol* 30:1278
  - Host B. 2000. *Eur J Clin Microbiol Infect Dis* 19:608-11
- Improves recovery of organisms from patients on antibiotics at time of specimen collection
  - Von Essen R. 1997. *Scand J Rheumatol* 26:293

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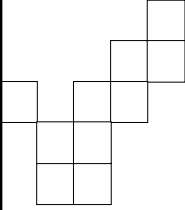
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## Urine Specimens

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## Urinary Tract Infections

- Urinary tract infections- most common bacterial infectious disease
- Urines-- most common sample type for culture
- Microbiologic diagnosis of UTIs has been impacted by several factors:
  - Changes in criteria for defining significant bacteriuria
  - Laboratory consolidation
  - Emergence of resistant organisms
  - Increases in numbers of immunocompromised pts.
  - Changes in technological advancements

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## UTI:Definitions

- UTI: presence of microorganisms in urine
- Symptomatic UTI: presence of clinical features
- Asymptomatic UTI:absence of symptoms in setting of critical numbers of potential uropathogens; local host response usually present

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## Criteria for Defining Significant Bacteriuria

- Symptomatic women
  - $\geq 10^2$  coliforms/mL or  $\geq 10^5$  non-coliforms/mL
- Symptomatic men
  - $\geq 10^3$  CFU bacteria/mL
- Asymptomatic individuals
  - $\geq 10^5$  CFU bacteria/mL
- Catheterized patients
  - $\geq 10^3$  CFU bacteria/mL
- Any growth on suprapubic aspirate or intraoperatively obtained sample

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## Etiology of Urinary Tract Infections

Figure 1 Etiology of UTI in Men

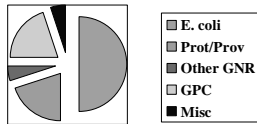
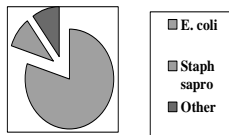


Figure 2 Etiology of UTI in Women



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## Complicated/Hospital Acquired UTIs: Etiology

- |                        |                                      |
|------------------------|--------------------------------------|
| ■ <i>E. coli</i>       | ■ Coagulase negative staphylococci   |
| ■ <i>Klebsiella</i>    | ■ Enterococci                        |
| ■ <i>Proteus</i>       | ■ <i>Corynebacterium urealyticum</i> |
| ■ <i>Providencia</i>   | ■ Yeasts                             |
| ■ <i>Serratia</i>      |                                      |
| ■ <i>Enterobacter</i>  |                                      |
| ■ <i>Acinetobacter</i> |                                      |
| ■ <i>Pseudomonas</i>   |                                      |

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## Urine Cultures: Indications

- Culture is not always necessary in women with dysuria, pyuria
- Cultures are indicated in the following situations:
  - ☐ complicated or uncertain clinical features
  - ☐ UTI in past 3 weeks indicating possible relapse
  - ☐ symptoms for more than 7 days
  - ☐ recent hospitalization or catheterization indicating possible nosocomial infection
  - ☐ pregnancy
  - ☐ diabetes

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## Urine Culture Specimen Collection

- Routine midstream clean catch urine specimen
  - ☐ Appropriate instructions must be provided to females, specimen should be collected after thoroughly cleansing the urethral opening with soap
  - ☐ Cleansing not necessary in males
  - ☐ Specimen should be a clean caught, mid-stream, early morning specimen
  - ☐ Transport urine specimens in leakproof containers within an one hour of collection
  - ☐ Refrigerate or use boric acid transport system if it cannot be transported and plated within an hour
  - ☐ Include pertinent patient information

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## Urine Culture Specimen Collection



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## Urine Culture Specimen Collection

- Straight catheterization or suprapubic aspiration
  - performed under strict aseptic conditions
  - to determine the significance of borderline counts in repeated clean catch midstream specimen
  - to diagnose anaerobic bacteriuria

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## Urine Culture Specimen Collection

- Chronic bladder catheterization
  - clean collection port of catheter tubing carefully with 70% alcohol
  - aspirate specimen using a sterile syringe and dispense in a sterile container

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## Urine Culture Specimen Rejection Criteria

- Urine collected from bedpan or urinal or collection bag
- Frozen specimen
- 24 h. collections for microbiologic culture
- A Foley catheter tip

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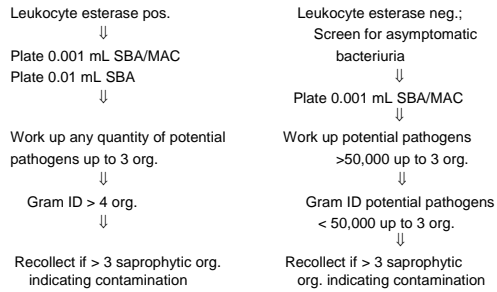
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## Urine Culture Algorithms: Women



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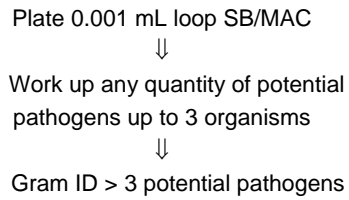
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## Urine Culture Algorithms: Men



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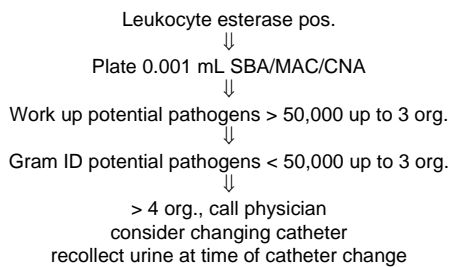
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## Urine Culture Algorithms Indwelling Catheters



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## Media, Inoculum and Incubation

- Media
  - 5% sheep blood agar
  - MacConkey agar or EMB
  - Colistin-nalidixic acid agar (CNA)
- Inoculate using calibrated loop method
- Incubate at 35-37° C for 18-24 h; 48 h for the following organisms
  - Yeasts
  - *Corynebacterium urealyticum*
  - Other coryneforms
  - *P. aeruginosa*

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## Differential Media

- Dip inoculum methods
- Chromogenic media
- Potential advantages
  - Ease in differentiating mixed flora
  - Eliminate need for subculture when performing susceptibility testing
  - Control of swarming *Proteus*
- Limitations
  - Few studies have performed in depth cost-analysis
  - Accuracy for detection of non-enterococcal, Gram-positive cocci and yeast is variable and needs more study



CHROMagar™ Orientation

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Questions?

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